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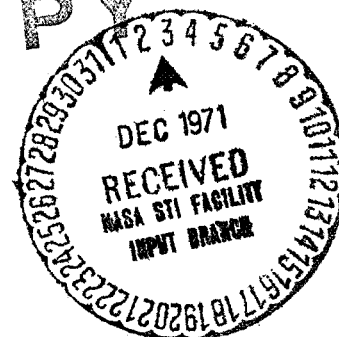
N71-36468

FOURTEENTH SUMMARY REPORT OF PROGRESS

Services Provided in Support of the Planetary Quarantine Requirements
of the
National Aeronautics and Space Administration

GERMICIDAL ACTIVITY OF ETHYLENE OXIDE

CASE FILE
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by the
U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
Health Services and Mental Health Administration
National Communicable Disease Center
Microbiological Control Section, Bacterial Diseases Branch
Epidemiology Program

R-137

W-13-062

October 1969

Atlanta

Germicidal Activity of Ethylene Oxide

Challenge of Environmental Bacteria with Gaseous Ethylene Oxide --

Sampling efforts have continued using millipore-filters to collect naturally occurring airborne microflora (Twelfth Summary Report of Progress). Filters with a 0.8 micron pore size were exposed to natural extramural contamination in Atlanta, Georgia and Phoenix, Arizona. A total of 9600 ft³ air was sampled with 144 filters operated for six- or seven-hour sampling periods at 10 ft³/hr/filter. Natural contamination from 7620 ft³ of air (on 114 filters) was exposed to ethylene-oxide gas while the collection from 1980 ft³ of air (on 30 filters) served as control samples.

Filters were aseptically removed from filter holders and placed in clean, sterile Petri dishes. Organisms on the filters were then exposed to ethylene-oxide gas at 1000 mg/L for 18 hours at 120 F after prehumidification at 50% RH for one hour. Control filters were subjected to identical conditions of temperature and humidity but not to gaseous ethylene oxide. Following exposure of filters, both the control and gas-exposed filters were placed on an agar media and incubated at 37 C. Control and exposed filters incubated aerobically were cultured on Tryptic soy agar in 5% CO₂, and filters incubated anaerobically were cultured on anaerobic agar in 10% CO₂ and 90% nitrogen. All control samples were incubated for 48 hours. Samples exposed to gaseous EtO were cultured similarly to the control samples; however, the incubation time was extended to 14 days. Following incubation, all filters were stained with Ponceau S dye for one hour and the excess dye

was removed from the filter matrix by overnight diffusion on 3% agar at room temperature. Then, surface colonial growth was counted and recorded with the aid of a binocular dissecting microscope.

Viable particle counts on the control filters ranged from 10 to 122 per filter with a mean of approximately 80 as determined by aerobic methods of cultivation. Anaerobic cultivation produced counts ranging from 0 to 6 viable particles per filter with a mean of approximately 2 per filter. Filters exposed to ethylene oxide and incubated for 14 days have not demonstrated survival of microorganisms.

Approximately 5×10^3 particles bearing viable material on millipore filters were exposed to ethylene-oxide gas. Anticipated filter counts were approximately 3×10^4 viable particles for the sample size collected, based on limited comparisons with concurrent samples taken using Reyniers air samplers and incubated under conditions similar to those used with the filters. In an attempt to increase the counts, sample times will be reduced to one- and two-hour periods. This procedure will reduce extraneous antagonistic chemical air pollution that is likely collected on the filter and may be a cause of numerical reductions in control counts. Damage to organisms by dessication during long sampling runs, which may also be an important factor in the low counts on filters, will also be reduced with the shorter runs.

Exposure of Anaerobic Spores to Gaseous Ethylene Oxide -- Studies on
experimental systems to obtain large numbers of anaerobic spores of Cl. perfringens have been continued (Eleventh Summary Report of Progress). High concentrations of washed spores of the NCTC 8238 strain in aqueous suspension have been added to sterile, dry house dust; blended; and dispensed for drying in controlled-humidity chambers regulated to maintain 90 F at 2 to 4% RH. Assays have been conducted to determine when the spore-laden dust stabilized in the "dry" state to a survival plateau. Aerobic spores of Bacillus globigii added to sterile dust and then dried have demonstrated a well-defined population plateau at 3 to 4 weeks after about a log reduction in numbers during drying. In contrast, following similar procedures of spore harvest, storage, dust seeding, and drying, numerous attempts have not produced a stable population of Cl. perfringens in house dust inoculated with 2×10^6 to 7×10^8 spores per ml; population assays performed on spore-laden dust indicated continuous reductions in initial counts exceeding four logs after four weeks of drying. The cause of the unfavorable decline is unestablished. In an attempt to alleviate the current difficulty of die-off of spores during drying in dust-spore mixtures, efforts are being directed toward preparation of high-concentration stock spore suspensions to be pre-dried prior to addition of dried dust.